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Seed Effects on Gibberellin Metabolism in Pea Pericarp[1,2]

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ABSTRACT

Pea fruit (*Pisum sativum* L.) is a model system for studying the effect of seeds on fruit growth in order to understand coordination of organ development. The metabolism of ^{14}C-labeled gibberellin A_{12} (GA_{12}) by pea pericarp was followed using a method that allows access to the seeds while maintaining pericarp growth in situ. Identification and quantitation of GAs in pea pericarp was accomplished by combined gas chromatography-mass spectrometry following extensive purification of the putative GAs. Here we report for the first time that the metabolism of [^{14}C]GA_{12} to [^{14}C]GA_{19} and [^{14}C]GA_{20} occurs in pericarp of seeded pea fruit. Removal of seeds from the pericarp inhibited the conversion of radiolabeled GA_{19} to GA_{20} and caused the accumulation of radiolabeled and endogenous GA_{19}. Deseeded pericarp contained no detectable GA_{20}, GA_1, or GA_8, whereas pericarp with seeds contained endogenous and radiolabeled GA_{20} and endogenous GA_1. These data strongly suggest that seeds are required for normal GA biosynthesis in the pericarp, specifically the conversion of GA_{19} to GA_{20}.

In pea (*Pisum sativum* L.), normal pod (pericarp) growth requires the presence of seeds (4); developing seeds contain high levels of GAs[3] (11, 12); and the requirement for seeds can be replaced by application of the plant hormone, GA (4, 17). Based on these observations, it has been assumed that the GAs biosynthesized by seeds are transported to the pericarp and regulate pericarp growth (6). However, Sponsel (17) proposed an alternative hypothesis that seeds may promote pericarp growth by maintaining GA biosynthesis in the pericarp. To test these hypotheses, our group initiated studies on GA biosynthesis in pea pericarp. The early 13-hydroxylation pathway is known to occur in pea (9): $GA_{12} \rightarrow GA_{53} \rightarrow GA_{44} \rightarrow GA_{19} \rightarrow GA_{20} \rightarrow GA_1 \rightarrow GA_8$. We have previously shown that pericarp tissue metabolizes [^{2}H]GA_{53} to [^{2}H]GA_{20} and [^{2}H]GA_1, but only when the pericarp is intact and attached to the plant (13). This report describes the first evidence that supports the hypothesis that GA biosynthesis from GA_{12} occurs in the pericarp and is regulated remotely by the presence of seeds.

MATERIALS AND METHODS

Plant Material and Treatments

Seeds of pea (*Pisum sativum* L.) line I_3 (Alaska-type) were grown as described by Maki and Brenner (13). Flowering began approximately 30 d after planting. Day 0 of fruit development was defined as the time when the petals were fully reflexed.

The following surgical procedure was used to split the pericarp and remove fertilized ovules (seeds). The pericarp of ovaries (pericarp + seeds) were split down the dorsal suture, either without disturbing the seeds (SP treatment), or alternatively, the seeds were removed from the SP (deseeded) using forceps (SPNS treatment). High humidity was maintained by enclosing the pericarp in clear plastic bags. Terminal apical meristems of plants were intact and ovaries were fertilized in all treatments.

To measure the effect of seed number on pericarp elongation, 2 DAA pericarp of ovaries were split as described above or left intact. Seeds were removed to obtain a final seed number of 0, 2, 4, 6, or 8 (all). The seeds removed were most distal to the midpoint of the pericarp, the remaining seeds being attached to the longitudinal midportion of the pericarp.

To measure the effect of GA_3 and STS on the metabolism of [^{14}C]GA_{12} in pea pericarp, 3- or 4-DAA pericarp were either split as described above or left intact. Pericarp were treated with a 10-s dip of either GA_3 (7 μM) or STS (1 or 10 mM) in 0.1% aqueous Tween 80 immediately and 20 h after the surgical treatment. Control treatments were dipped in a 0.1% aqueous Tween 80 solution. Splitting of the pericarp and seed removal were completed 24 h prior to radiolabel application. In growth measurement experiments, pericarp were treated daily with a 10-s dip in either GA_3 (7 μM) or STS (1 or 10 mM) in 0.1% aqueous Tween 80.

Labeled GAs

[^{14}C]GA_{12} was biosynthesized from *R,S*-[4,5-^{14}C]mevalonic acid (110 μCi/μmole) using a cell-free system of pumpkin endosperm as described by Birnberg et al. (2), except that prior to HPLC purification, the reaction mixture containing the radioactive products was purified by charcoal chromatography (18). The specific radioactivity of [^{14}C]GA_{12} was determined from its mass spectra to be 180.5 μCi/μmol using the method described by Bowen et al. (3). Deutero- and protio-GA_1, GA_8, GA_{19}, and GA_{20} were purchased from Dr. L.N.

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[2] These results were originally presented at the 14th International Conference on Plant Growth Substances Meeting held in Amsterdam, The Netherlands, in July 1991, and parts will appear in the proceedings from this meeting.

[3] Abbreviations: GA, gibberellin; SP, split pericarp; SPNS, split pericarp no seeds; DAA, days after anthesis; STS, silver thiosulfate; SIM, selective ion monitoring; KRI, Kovat's retention index.

Mander (Research School of Chemistry, Australian National University). [^{14}C]GA_{19} and [^{14}C]GA_{20}, used as standards for TLC, were a gift from Dr. Peter Davies (Cornell University).

Tissue Incubation and Extraction

[^{14}C]GA_{12} was applied to the inside surface (endocarp) of pericarp at 3 or 4 DAA; the ovaries remained attached to the plant. Pericarp tissues (seeds were removed if present) were harvested onto dry ice 24 h after [^{14}C]GA_{12} application and stored at −80°C until extraction.

One (for TLC), 40 (SP treatment; 11.4 g), or 39 pericarp (SPNS treatment; 3.9 g) per sample were homogenized in cold 80% aqueous methanol (10 mL for single pericarp samples and 60 mL for 40 pericarp samples) containing 10 mg/L butylated hydroxytoluene using a Polytron homogenizer (Brinkman Instruments, Westbury, NY). To each of these larger scale extracts, [^{2}H]GA_1, [^{2}H]GA_8, [^{2}H]GA_{19}, and [^{2}H]GA_{20} (100 ng each) were added at homogenization as internal standards for GC-MS-SIM. The tissue homogenates were extracted for 12 to 16 h by shaking gently or stirring in darkness at 4°C. The extracts were centrifuged for 30 min at 10,000*g* and the pellet was washed twice. The supernatant was evaporated to dryness (single ovary samples) or to the aqueous phase (pericarp samples for GC-MS-SIM) in silylated glass vials using a SpeedVac concentrator (Savant, Farmingdale, NY) without supplemental heating.

Chromatography

The extracts of single ovary samples were resuspended in 50 μL of ethanol followed by 800 μL of 0.1 N acetic acid, passed through 0.45-μm nylon filters, and injected onto a 4.6 × 150 mm Spherisorb C_{18} column (5 μm; Phase Sep, Norwalk, CT). Radioactivity in the effluent was monitored with a flow-through Packard Trace 7140 radiochemical detector in the heterogeneous mode (Downers Grove, IL). The samples were eluted at a flow rate of 1 mL/min using a linear gradient elution of 0.1 N acetic acid (solvent A) with 0.1 N acetic acid in acetonitrile (solvent B) as follows: 100% A in 1 min, gradient to 80% A in 2 min, gradient to 65% A in 15 min, gradient to 25% A in 15 min, gradient to 0% A (100% B) in 2 min, and isocratic 100% B for 5 min (14). This is referred to as HPLC System 1.

The fraction containing GA_{19} and GA_{20} (15.8 min) was collected and dried under a stream of N_2. The residue was resuspended in 50 μL of 80% aqueous methanol and spotted (2000–4000 dpm/sample) onto silica gel TLC plates (Unisil G or Silica GF; Analtech, Newark, DE), and the plates were developed 15 to 17 cm in an ethyl acetate:chloroform:acetic acid (90:30:1) solvent system. After development, radioactivity on the TLC plates was monitored using an Ambis Systems (San Diego, CA) gel scanner (16 h exposure/plate).

The pericarp aqueous extracts for GC-MS-SIM were filtered through silylated glass wool. After adjusting the pH to 8.0 with NH_4OH (0.1 N), the extracts were partitioned against *n*-hexane (5 mL) four times. The aqueous fraction was then adjusted to pH 3.0 with HCl (0.1 N) and partitioned against ethyl acetate (5 mL) five times. The combined ethyl acetate extracts were partitioned against 5% (w/v) aqueous $NaHCO_3$ (5 mL) four times. The combined $NaHCO_3$ extracts were adjusted to pH 3.0 and partitioned against ethyl acetate (5 mL) four times. The ethyl acetate extracts were combined and evaporated to dryness under vacuum. The residues were chromatographed on HPLC using the 5-μm Spherisorb C_{18} column and HPLC Solvent System 1 as described above. Fractions eluting at the retention time of GA_8 (7.8 min), GA_1 (9.8 min), and GA_{19} and GA_{20} (both 15.8 min) were collected and dried under a stream of N_2. These fractions were rechromatographed on a 4.6 × 150 mm Nucleosil 10 μm $N(CH_3)_2$ (Macherey-Nagel; Duren, Germany) column isocratically eluted at 1 mL/min with 0.1 N acetic acid in methanol. Fractions eluting at the retention time of GA_1 (6.2 min), GA_{20} (6.4 min), GA_8 (6.6 min), and GA_{19} (7.6 min) from the $N(CH_3)_2$ column were collected and dried under a stream of N_2. These fractions were rechromatographed on a 3.9 × 150 mm Nova-pak 4-μm C_{18} (Waters) column at a flow rate of 1 mL/min using a 40-min linear gradient of 40 to 90% methanol in 0.1 N acetic acid (8). Fractions eluting at the retention time of GA_8 (2.2 min), GA_1 (3.4 min), GA_{20} (10.2 min), and GA_{19} (13.0 min) were collected and dried under a stream of N_2. Samples were methylated using diazomethane and rechromatographed on the Spherisorb C_{18} column using the HPLC Solvent System 1 gradient (retention time: GA_8, 9.8 min, GA_1, 13.0 min, GA_{20}, 23.2 min, and GA_{19}, 27.0 min).

GC-MS-SIM

Methylated samples containing putative GAs were converted to their methyl ester-trimethylsilyl ether derivatives (13). Mass spectral analyses of derivatized samples were performed using a Hewlett-Packard model 5890 gas chromatograph interfaced to a Hewlett-Packard model 5970 Mass Selective Detector as described by Maki and Brenner (13).

For identification and quantitation of endogenous and radiolabeled GAs, five (GA_{19}) or three ions (GA_1, GA_8, GA_{20}) for each protio-, deutero- (internal standard), and ^{14}C-labeled (M + 16) GAs were measured simultaneously. The protio- and deutero-ions monitored were corrected for donation of natural isotopes to the peak area. For GA_{19} and GA_{20}, a separate calibration curve of peak area ratio versus molar ratio of[^{2}H]GA/protio-GA was constructed (10). Using the corrected peak area and the calibration curve, the total amounts of protio- and ^{14}C-labeled GA_{19} and GA_{20} were calculated. Protio-GA_1 was not available for construction of a calibration curve; therefore, the isotope dilution equation used by Bandurski and Schulze (1) was used for this calculation. For calculation of endogenous GAs, contributions from the protio-GAs derived from the [^{14}C]GA_{12} (180 μCi/μmol) added to the tissue were subtracted from the calculated total protio-GA value. The most prominent ion measured (usually M^+) was used for quantitation, and the calculated value was checked for reproducibility using the second most prominent ion. KRI with fragmentation data were used for confirmation of GA identity. The detection limit for GA by GC-MS-SIM was approximately 0.1 ng.

RESULTS

Splitting the pericarp while it remained attached to the plant provided access to the seeds while maintaining seed and pericarp development to maturity. Mature seeds from SP

germinated and produced normal plants. Minimal pericarp damage occurred on opening of the ovary, and the pericarp with seeds continued to elongate, reaching 82% of the length of the intact pericarp (Table I). However, fresh and dry weights were less than half that of the intact ovaries at 15 DAA (Table I).

Seeds were required for continued pericarp elongation. Removal of all seeds 2 DAA resulted in senescence and abscission of the pericarp within 5 d after seed removal (Table I). When seed number was manipulated to obtain two, four, six, or eight seeds per pericarp, pericarp length and weight were proportional to seed number (Table I). Some seeds aborted; hence, the number of surviving seeds 15 DAA was less than at 2 DAA.

Metabolism of [^{14}C]GA_{12} by pea pericarp was investigated by applying [^{14}C]GA_{12} to the inside pericarp wall (endocarp) of ovaries with and without fertilized ovules (seeds). Pericarp were turgid and green, and the tissue integrity remained intact throughout the period of incubation. Within 24 h, pericarp with seeds metabolized [^{14}C]GA_{12} to putative [^{14}C]GA_{19} and [^{14}C]GA_{20} as determined following HPLC and TLC (GA_{19} R_f 0.33–0.34; GA_{20} R_f 0.67–0.69; Table II). In contrast, when the seeds were removed 24 h prior to [^{14}C]GA_{12} application, putative [^{14}C]GA_{19}, but no [^{14}C]GA_{20}, was detected in the extracts of the pericarp tissue (Table II; TLC sensitivity limit was 6 dpm above background). GA_{44} also cochromatographs with GA_{19} and GA_{20} (15.8 min) in HPLC Solvent System 1 using the C_{18} Spherisorb column. Therefore, the presence of putative [^{14}C]GA_{44} was also monitored on the TLC (R_f 0.47) plates. Little or no putative [^{14}C]GA_{44} was detected in pericarp samples after the 24-h incubation period (data not shown).

To test if wound ethylene produced following seed detachment was involved in inhibition of the conversion of GA_{19} to GA_{20}, pericarp were dipped in solutions of STS (1 or 10 mM), an inhibitor of ethylene action. Treatment with STS (10 mM) did not overcome the inhibition of conversion of putative [^{14}C]GA_{19} to [^{14}C]GA_{20} in the deseeded pericarp (Table III). Similar results were obtained using STS at 1 mM (data not shown). Three of five deseeded pericarp treated with STS (1 mM) lost tissue integrity within 8 d after seed removal; the remaining pericarp grew significantly less than the SP with seed control (Table IV). In general, deseeded pericarp treated with STS were greener than deseeded controls, abscission did not take place within 12 DAA, loss of tissue integrity was delayed but not prevented, and conversion of putative [^{14}C]GA_{19} to putative [^{14}C]GA_{20} did not occur (Tables III and IV).

Because pea seeds contain several active GAs, including GA_3 (6), seed-derived GAs may regulate GA metabolism in the pericarp. Conversion of putative [^{14}C]GA_{19} to putative [^{14}C]GA_{20} was not observed when deseeded pericarp were treated with 7.2 μM GA_3 (Table II), a concentration that stimulated pericarp growth (Table IV).

In preliminary findings (16), we reported the presence of putative [^{14}C]GA_{20} (HPLC data) in pericarp both with and without seeds. Subsequently, we found that [^{14}C]GA_{53} conjugate is produced in relatively high amounts and cochromatographs with [^{14}C]GA_{20} in the HPLC system 1. The presence of this contaminant in the preliminary experiments masked the changes in [^{14}C]GA_{20} and demonstrated the need for another chromatography step following HPLC for the [^{14}C]GA metabolite assays. TLC was chosen as the second method because the high detection sensitivity of the TLC scanner for [^{14}C]GA metabolites (approximately 10 times higher than the HPLC radiochemical flow-through detector) was required for the one ovary per assay sample size. The TLC assays (Tables II and III) permitted semiquantitative analysis of GA_{12} metabolism in our experimental system.

Subsequently, pericarp were bulked after incubation with [^{14}C]GA_{12}, extracts were purified, and GAs were unequivocally identified and quantified by GC-MS-SIM using ^{2}H-labeled GAs as internal standards. ^{14}C-labeled and endogenous GA_{19} were identified in SP both with (SP treatment) and without seeds (SPNS treatment; Table V). The deseeded pericarp contained 3.1 times more endogenous GA_{19} (13.7 ng/g fresh weight) and 1.9 times more [^{14}C]GA_{19} (11.5 ng/g fresh weight) than pericarp with seeds (4.4 ng/g fresh weight

Table I. *The Effects of Seed Removal Following Splitting of Pericarp 2 DAA on Pericarp Length, Pericarp Weight, and Remaining Seed Weight in Pea 15 DAA (Cultivar I_3 Alaska Type)*

	No. of seeds (2 DAA)	Pericarp[a]			Seeds	
		Length	Fresh wt	Dry wt	No. of seeds (15 DAA)	Fresh wt per seed
		mm	*g*	*g*		*mg*
	0	Abscised[b]				
	2	40.4a	0.8a	0.10a	1.4a	187a
	4	45.7b	1.1a, b	0.14b	2.3b	208a
	6	48.4b	1.2b	0.16c	3.0c	163a
	8	55.6c	1.7c	0.23d	4.3d	179a
		0.730**	0.653**	0.631**	0.812**	−0.111 (n.s.)
Intact pericarp[d]	7–8	68.1 ± 1.6	3.7 ± 0.3	0.66 ± 0.05	5.6 ± 0.2	303 ± 9.7

[a] Pericarp split 2 DAA, when average length = 13.7 ± 0.4 mm. [b] Within column, means (10 replicates) followed by the same letter are not significantly different from one another by Duncan's multiple range test, $P < 0.05$. [c] Linear correlation coefficients (r) of seed number (2 DAA) versus pericarp length, weight, and seed number, and seed weight at 15 DAA. [d] Data obtained in separate experiment; ± SE. **, Significant at $P = 0.01$. n.s., Not significant at $P = 0.05$.

Table II. *Effects of Pericarp Splitting, of Seed Removal, and of Application of GA_3 on Biosynthesis of Putative [^{14}C]GA_{19} and [^{14}C]GA_{20} (Percent of Radioactivity on TLC Plate) in Pea Pericarp Incubated With [^{14}C]GA_{12}*

Treatment[a]	Pericarp	Seeds	Percent [^{14}C] from TLC Plate at R_F of			
			GA_{19}		GA_{20}	
			dpm	%	*dpm*	%
Experiment 1, one ovary per sample; [^{14}C]GA_{12} applied to 3 DAA ovaries						
None	Intact	+	561[b]	12.0 ± 4.6[c]	215	8.5 ± 2.7
None	Split	+	509	16.7 ± 4.6	200	9.5 ± 2.7
None	Split	−	152	7.9 ± 1.2	N[d]	
GA_3 (7.2 μM)	Intact	+	388	24.0 ± 7.1	115	7.1 ± 2.1
GA_3 (7.2 μM)	Split	+	124	8.1 ± 5.7	118	9.6 ± 0.8
GA_3 (7.2 μM)	Split	−	252	13.3 ± 1.3	N	
Experiment 2, one ovary per sample; [^{14}C]GA_{12} applied to 4 DAA ovaries						
None	Intact	+	840	12.9 ± 2.5	431	7.2 ± 2.7
None	Split	+	343	7.1 ± 2.6	95	2.0 ± 1.0
None	Split	−	109	1.9 ± 0.7	N	
GA_3 (7.2 μM)	Intact	+	866	28.6[e]	281	9.3[e]
GA_3 (7.2 μM)	Split	+	105	5.3 ± 0.3	90	4.7 ± 2.8
GA_3 (7.2 μM)	Split	−	161	7.9 ± 1.3	N	
Experiment 3, pooled samples (39 or 40 ovaries per treatment); [^{14}C]GA_{12} applied to 3 DAA ovaries						
None	Split	+		15.8		17.7
None	Split	−		15.2		N

[a] Hormone treatments applied 28 and 4 h prior to application of [^{14}C]GA_{12}. [b] Average dpm. [c] SE of three separate pericarp; exception, GA treated, mean of two pericarp. [d] N, Not detected. [e] Single pericarp sample.

endogenous; 6.0 ng/g fresh weight ^{14}C-labeled; Table VI). In contrast, pericarp with seeds contained both endogenous GA_{20} (5.3 ng/g fresh weight) and [^{14}C]GA_{20} (1.8 ng/g fresh weight), whereas deseeded pericarp contained neither (Tables V and VI). Endogenous GA_1 (1.0 ng/g fresh weight) was detected only in pericarp with seeds, whereas [^{14}C]GA_1 was not detected in pericarp with or without seeds (Tables V and VI). Neither endogenous nor ^{14}C-labeled GA_8 were detected in deseeded pericarp (Tables V and VI); GA_8 in pericarp with seeds was below the GC-MS-SIM detection limit due to low sample recovery. As a result of decreased pericarp growth after seed removal, endogenous and ^{14}C-labeled GA_{19} values expressed per ovary are higher for pericarp with seeds (4.2 and 1.7 ng/ovary, respectively) than for deseeded pericarp (3.3 and 1.2 ng/ovary, respectively).

DISCUSSION

The presence of fertilized ovules (seeds) is required for pericarp integrity and elongation as demonstrated by killing (4, 17) or removing (Table I) seeds. Surgically opening the pericarp allowed the manipulation of seeds while maintaining viable seed and elongating pericarp (Table I). Pericarp length, fresh weight, dry weight, and final seed number were all positively correlated with initial seed number (Table I). Therefore, pericarp length is determined in part by the number of seeds present in the ovary.

Using the SP technique, we were able to follow metabolism of [^{14}C]GA_{12} in pericarp with and without seeds. Our data confirm and extend the results of Maki and Brenner (13) that pea pericarp metabolizes GAs by the 13-hydroxylation path-

Table III. *Effects of STS on Biosynthesis of Putative [^{14}C]GA_{19} and [^{14}C]GA_{20} (Percent of Radioactivity on TLC Plate) in Pea Pericarp Incubated with [^{14}C]GA_{12}*

One ovary per sample; two pericarp per treatment; [^{14}C]GA_{12} applied to SP 4 DAA.

Treatment[a]	Seeds	Percent [^{14}C] from TLC Plate at R_F of			
		GA_{19}		GA_{20}	
		dpm	%	*dpm*	%
None	+	211[b]	9.4 ± 1.0[c]	250	11.0 ± 5.4
None	−	54	2.4 ± 2.3	N[d]	
STS (10 mM)	+	97	3.8 ± 0.6	274	10.6 ± 0.0
STS (10 mM)	−	306	11.3 ± 8.0	N	

[a] STS treatment applied 28 and 4 h prior to application of [^{14}C]GA_{12}. [b] Average dpm. [c] Standard error of mean. [d] N, Not detected.

Table IV. *Effects of Pericarp Splitting, Seed Removal, and Treatment With GA_3 or STS on Growth of Pea Pericarp*

Treatment	Pericarp[a]	Seeds	n[b]	Length	Fresh Wt	Dry Wt
				mm	*g*	*g*
Experiment 1[c]						
Control	Intact	+	5	64.0 ± 2.1[d]	2.64 ± 0.41	0.41 ± 0.08
Control	Split	+	4	49.5 ± 5.7	1.52 ± 0.59	0.21 ± 0.09
STS (1 mM)	Split	+	5	40.8 ± 5.7	1.40 ± 0.50	0.21 ± 0.08
Control	Split	−	5	Abscised[e]		
GA_3 (7.2 μM)	Split	−	7	44.2 ± 2.3	0.85 ± 0.14	0.11 ± 0.02
STS (1 mM)	Split	−	5	30.0 ± 4.0[f]	0.17 ± 0.06	0.02 ± 0.01
Experiment 2[g]						
Control	Intact	+	9	61.7 ± 1.6	2.99 ± 0.20	0.50 ± 0.04
Control	Split	+	10	45.1 ± 1.0	1.48 ± 0.15	0.21 ± 0.02
STS (10 mM)	Split	+	9	39.1 ± 2.3	1.25 ± 0.18	0.17 ± 0.03

[a] Pericarp split 3 DAA. [b] Number of replicates. [c] Pericarp harvested 11 to 12 DAA. [d] SE. [e] All pericarp abscised within 5 d of seed removal. [f] Three of five pericarp flaccid, data are for remaining two pods. [g] Pericarp harvested 19 to 21 DAA.

way, $GA_{12} \rightarrow GA_{19} \rightarrow GA_{20} \rightarrow GA_1$. We have shown that [^{14}C]GA_{12}, a key intermediate in the GA biosynthesis pathway, is metabolized by pea pericarp to [^{14}C]GA_{19} and [^{14}C]-GA_{20} when the seeds are present and the fruit is attached to the plant. The higher levels of ^{14}C-labeled and endogenous GA_{19} found in deseeded pericarp when compared to pericarp with seeds (Table VI), coupled with the lack of endogenous and ^{14}C-labeled GA_{20} in deseeded pericarp, suggest that the conversion of GA_{19} to GA_{20} is greatly reduced or inhibited in the pericarp when the seeds are removed. The inhibition of conversion of GA_{19} to GA_{20} does not appear to be an effect of wound ethylene as a result of seed removal, because treatment with STS, an ethylene action inhibitor, delayed ethylene-related physiological processes such as tissue senescence and pericarp abscission, but did not overcome the inhibition of GA_{20} synthesis in deseeded pericarp (Tables III and IV). These data strongly suggest that the seed regulates GA biosynthesis in the pericarp, specifically conversion of GA_{19} to GA_{20}. Metzger and Zeevaart (15), Gianfagna et al. (7), and Gilmour et al. (8) provided convincing evidence that the conversion of GA_{19} to GA_{20} is an important step in external or environmental regulation (photoperiodic control) of GA metabolism in spinach (*Spinacia oleracea*).

Our estimates of endogenous GA_{20} (5.3 ng/g fresh weight) and GA_1 (1.0 ng/g fresh weight) are close to the values of 5.01 and 0.49 ng/g fresh weight found by Garcia-Martinez et al. (6) for GA_{20} and GA_1, respectively, in intact 4 DAA pericarp. However, our endogenous GA_{19} estimate (4.4 ng/g fresh weight) in SP with seeds was 6.8 times greater than that found by Garcia-Martinez's group (0.64 ng/g fresh weight) in intact 4 DAA pericarp. The higher levels of endogenous GA_{19} could be due to the splitting of the pericarp in our treatment, preferential metabolism of protio-GA over [^{14}C]GA added to the pericarp tissue, resulting in an overestimation of the endogenous GA_{19}, lack of contamination of GA_{19} in our mass spectra versus contamination interference of GA_{19} mass spectra (as mentioned by Garcia-Martinez et al. [6]), resulting in a low estimation of endogenous GA_{19} by Garcia-Martinez's group, or variability due to different growth conditions.

Table V. *GC-MS-SIM Data for Metabolites of [^{14}C]GA_{12} in Extracts of Pea Pericarp and for Endogenous and Protio-GA_{19}, -GA_{20}, and -GA_1*

STDS	Treatment	KRI	Ions (Percent Abundance)
Protio-GA_{19}		2687	434 (100) 374 (66) 375 (53) 402 (36) 462 (6)
	SP endogenous GA_{19}	2689	434 (100) 374 (72) 375 (69) 402 (37) 462 (4)
	SP [^{14}C]GA_{19}	2690	450 (100) 391 (44) 390 (93) 418 (46) 478 (6)
	SPNS endogenous GA_{19}	2687	434 (100) 374 (67) 375 (55) 402 (36) 462 (5)
	SPNS [^{14}C]GA_{19}	2686	450 (100) 391 (87) 390 (78) 418 (38) 478 (6)
Protio-GA_{20}		2579	418 (100) 375 (70) 403 (16)
	SP endogenous GA_{20}	2579	418 (100) 375 (76) 403 (17)
	SP [^{14}C]GA_{20}	2578	434 (100) 391 (46) 419 (23)
	SPNS endogenous GA_{20}	2575	418 (100) 375 (75) 403 (2)
Protio-GA_1		2733	506 (100) 491 (11) 447 (12)
	SP endogenous GA_1	2731	506 (100) 491 (18) 447 (11)

Table VI. *Quantitation of GAs in SP With and Without Seeds by GC-MS*[a]

	Concentration							
	GA_{19}		GA_{20}		GA_1		GA_8	
	[^{12}C][b]	[^{14}C]	[^{12}C]	[^{14}C]	[^{12}C]	[^{14}C]	[^{12}C]	[^{14}C]
	ng/g fresh wt							
Seeds intact	4.4	6.0	5.3	1.8	1.0	N[c]	–[d]	–[d]
Seeds removed[e]	13.7	11.5	N	N	N	N	N	N

[a] Pericarp split 2 DAA. [b] Estimate of endogenous GA content; total protio-GA minus contribution of protio-GA from [^{14}C]GA_{12} added to pericarp. [c] N, Not detected. [d] –, Not determined due to low sample recovery. [e] Deseeded 2 DAA.

[^{14}C]GA_1 was not detected in SP with seeds, probably because the concentration was below the GC-MS-SIM detection limit (a small amount of radioactivity in the SP sample eluted at the retention time of GA_1-methyl ester in the last step of purification prior to GC-MS-SIM). However, [^{14}C]GA_{20} was likely converted to [^{14}C]GA_1 in the pericarp because Maki and Brenner (13) found that [^{2}H]GA_{53} was metabolized to [^{2}H]GA_{20} and [^{2}H]GA_1 in intact 4-DAA pericarp. The possibility that conversion of GA_{20} to GA_{29} (an inactive GA_{20} catabolite) is stimulated by seed removal, resulting in the total depletion of GA_{20}, also seems unlikely, because GA_{19} accumulation in the pericarp parallels the loss of GA_{20} and GA_1 following seed removal.

Ingram and Browning (11) and Garcia-Martinez et al. (6) reported that the highest levels of GA_{20} (5–7 ng/g fresh weight) (6) in elongating pericarp occur very early after fertilization (0–4 DAA). We did not detect GA_{20} in the pericarp 48 h after seed removal, yet a significant amount of [^{14}C]GA_{20} was synthesized within a 24-h incubation period in pericarp with seeds. Therefore, the pool of endogenous GA_{20} was depleted within 48 h of seed removal, reflecting rapid turnover. Such tight regulation of GA metabolism may be an indication that GA metabolism in the pericarp is important for the production of GAs that are active in stimulating pericarp elongation.

Garcia-Martinez et al. (6) found that the total amounts of all GAs studied in the pericarp of fertilized pea ovaries 4 to 6 DAA were higher than those of the seeds (GA_1, 2–3 times; GA_8, 10–16 times; GA_{19}, 4–5 times; GA_{20}, 15–16 times; and GA_{29}, 23 times), except for GA_3 (0.14 to 0.3 times). In this report, we have shown that the pericarp can synthesize GA_{19} and GA_{20} from exogenous substrates in quantities comparable to the endogenous levels and those measured by Garcia-Martinez et al. (6). These data, together with the data that pea pericarp can synthesize [^{2}H]GA_1 from [^{2}H]GA_{53} (13), support the hypothesis that the pericarp can synthesize GAs in amounts sufficient to stimulate its own growth. However, because transport of GAs from seeds and vegetative plant parts to the pericarp may be occurring, further experiments are required to determine the relative contribution of GA synthesis in the pericarp to the total pericarp GA pool.

In our system, GA_3 is not a seed-derived signal for stimulation of conversion of GA_{19} to GA_{20}, because deseeded pericarp treated with GA_3 at 7 μM metabolized [^{14}C]GA_{12} to putative [^{14}C]GA_{19}, but not to putative [^{14}C]GA_{20} (Table II). GA_3 may be such a signal for stimulation of $GA_{12} \rightarrow\rightarrow GA_{19}$, however, because deseeded pericarp treated with GA_3 had significantly higher levels of putative [^{14}C]GA_{19} than did deseeded controls (Table II). This increase in putative [^{14}C]GA_{19} levels could also be a consequence of enhanced growth.

Garcia-Martinez et al. (6) found that the majority of GA_3 in the ovary (pericarp + seeds) occurred in the seeds during rapid ovary elongation (2–6 DAA). They hypothesized that GA_3 would be a prime candidate for seed regulation of pericarp growth. Although GAs may be transported from the seed to the pericarp, GA_3 is probably not the seed-derived signal directly regulating pericarp elongation. If it were, GA_3 should be the major or only active GA in the pericarp during its elongation. However, GA_1, which also stimulates pericarp elongation (17), occurs in higher levels in the pericarp than GA_3, and GA_1 is not considered to be a precursor of GA_3 (5). The effect of GA_3 could be indirect, promoting the metabolism of GA_{12} to GA_{19}, but not the conversion of GA_{19} to GA_{20}.

The concentrations of [^{14}C]GA_{19} and [^{14}C]GA_{20} found in pericarp with or without seeds were similar to those of endogenous GA_{19} and GA_{20} within the same treatment (Table VI). This is a strong indication that seed regulation of conversion of GA_{19} to GA_{20} in the pericarp occurs naturally and is not an artifact of [^{14}C]GA_{12} application to this tissue. In addition, if the majority of GA_{19} or precursor (GA_{53} or GA_{44}) is derived from the seed, the level of endogenous GA_{19} should decline in deseeded pericarp. Because this is not the case, transport of these GAs from the seed to the pericarp probably is not occurring appreciably during the period measured. Garcia-Martinez et al. (6) found that the level of endogenous GA_{19} and GA_{20} decreased from 0 to 4 DAA in unfertilized pea ovaries. Although these data are in general agreement with our own, a direct comparison cannot be made between unfertilized ovaries (treatment used by Garcia-Martinez et al. [6]) and fertilized ovaries with the seeds subsequently removed (treatment used in this report). In experiments comparing unfertilized to fertilized ovaries, the effect of fertilization must be taken into account when comparing levels of GAs in pericarp with seeds to pericarp without seeds (unfertilized ovaries). When using fertilized ovaries and removing the seeds surgically, the seed effect on GA levels and growth is not confounded by fertilization effects.

We have not only shown that pericarp have the capacity to metabolize [^{14}C]GA_{12} to [^{14}C]GA_{19} and [^{14}C]GA_{20}, but have

also presented evidence that the seed regulates GA biosynthesis in the pericarp, specifically, conversion of GA_{19} to GA_{20}. These data support the alternative hypothesis that the presence of seeds indirectly maintains GA production in the pericarp. A transmittable seed factor may be responsible for stimulating GA biosynthesis. It is also possible that the sink strength of the seeds is involved in maintaining pericarp GA biosynthesis. Using the SP growth system, we intend to further investigate the seed effect of conversion of GA_{19} to GA_{20} using [^{14}C]GA_{19} as a substrate to characterize the kinetics of this step. We also intend to investigate the nature of possible seed stimulatory factors and determine the importance of GA biosynthesis in the pericarp on the process of elongation of the pericarp.

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